Epidermal Growth Factor/Transforming Growth Factor Alpha Receptors and Psoriasis

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The abnormal growth and differentiation in psoriasis is reflected in the abnormal regulation of Epidermal Growth Factor/Transforming Growth Factor Alpha (EGF/TGFα) receptor metabolism. In psoriasis and other hyperproliferative skin conditions these receptors are persistently expressed throughout the interfollicular epidermis as long as the growth stimulatory signal persists. One of the first biochemical signs of effective therapy of psoriasis is the return of the EGF/TGFα receptor pattern toward the primarily basilar distribution seen in normal human adult skin. Whether the abnormal expression of TGFα in the involved skin induces the persistent expression of EGF receptors is not known nor is the signal that causes the increased production of TGFα. Studies to determine what factors regulate EGF receptor expression and TGFα induction may yield important new insights into the pathogenesis and therapy of psoriasis. *J Invest Dermatol 95:10S–12S, 1990*

One approach to studying growth regulatory mechanisms in psoriasis is to use well-studied proteins capable of regulating normal human keratinocyte proliferation and differentiation, i.e., EGF, TGFα, and their common, but specific membrane receptor [1–3]. EGF is a small (53 amino acid) protein that directly stimulates growth and differentiation in a number of epithelial tissues, stimulates the growth of mesenchymal and endothelial cells, and has multi-functional effects such as ion secretion, muscle contraction, and cell movement [1–3]. For example, in vivo EGF/urogastrone stimulates growth of lung, corneal, tracheal, and GI tract epithelium and inhibits gastric acid secretion [3]. The primary sequence of EGF, urogastrone, TGFα, and structurally related proteins (Fig 1) has been determined, and many of them have been cloned [2]. EGF and TGFα are present in human platelets, and EGF is found in all body fluids. TGFα has overall but limited homology to EGF and is antigenically related, binds to the EGF receptor, and in some systems has different or more profound effects than EGF [3,4]. Because these and other less well-defined proteins, such as vaccinia virus growth factor (VVGF), are structurally related to EGF and bind to the EGF/TGFα receptor, this receptor may be involved in a number of seemingly unrelated diseases [2].

All known effects of EGF and TGFα are mediated by their specific receptor, and if that receptor is mutated as in the v-erb B oncogene it causes cancer or abnormal proliferation such as with psoriasis [2]. Therefore, errors in EGF/TGFα receptor metabolism such as point mutations and internalization, degradation, phosphorylation, dephosphorylation, or glycosylation defects may cause human skin diseases. Abnormalities in the EGF/TGFα receptor can occur at a number of sites, because it is a single-chain glycoprotein of approximately 170,000 kDa containing an extracellular EGF binding site, a small transmembrane portion, and a cytoplasmic intrinsic EGF stimulated tyrosine kinase, along with its phosphate acceptor sites. When EGF or related proteins bind to the extracellular portion of the EGF/TGFα receptor, the receptor autophosphorylates itself or other biologically active proteins on specific tyrosine residues to transduce extracellular signals regulating cell proliferation/differentiation. If growth factor binding is increased or decreased, or this signal is altered by known (PDGF, TGFβ) or unknown factors, then the epidermis may have abnormal growth and/or differentiation.

EGF/TGFα Receptor Localization and Metabolism The EGF/TGFα receptor is almost ubiquitous in non-hematopoietic cells. These localization data based on the binding of radioactive ligands and anti-receptor antibodies correspond well to the localization of mRNA for EGF, TGFα, and their receptor [5]. Immunoreactive and ^{125}I-EGF binding receptors were found in cell membranes of dividing and non-dividing epithelial and mesenchymal cells [6]. The receptors are expressed in a developmentally regulated manner in embryonic human skin and are differentially expressed in hair follicle formation.* Even in embryonic and neonatal skin, receptor expression appears to be under regulatory control that can be modulated and/or reversed.* In rapidly growing newborn foreskin epidermis the EGF/TGFα receptors appear to be expressed in all layers of the epidermis.* At a later time the more adult pattern with receptors primarily confined to the basal cell layers is seen. When the adult pattern is seen in the newborn period, as it is in restrictive dermopathy, this distribution is abnormal. Therefore, it is important to know that the normal patterns of receptor expression are important for both the site and the developmental age. In normal adult human epidermis EGF/TGFα receptors, as detected by binding of ^{125}I-EGF, are found in the highest concentrations on basal keratinocytes and decrease in number as keratinocytes differentiate to become the normal stratum corneum [6]. This ^{125}I-EGF binding pattern in human epidermis has been confirmed, but there may be slight variations in the pattern of immunoreactive EGF/TGFα re-

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Abbreviations:

EGF/TGFα: epidermal growth factor/transforming growth factor alpha

PKG: protein kinase c

* Nanney et al: J Invest Dermatol (submitted).

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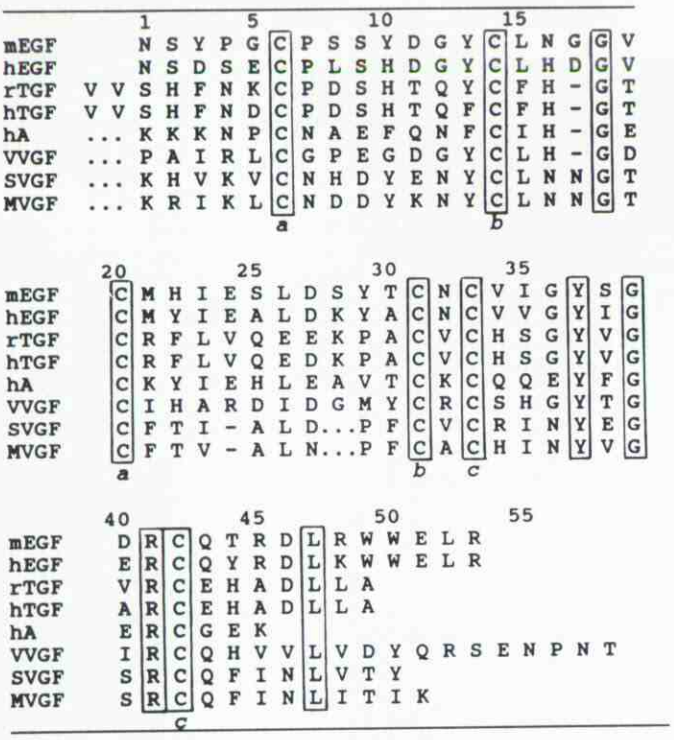

Figure 1. Comparison of peptides having EGF activity is shown. The sequences of mouse EGF (mEGF), human EGF (hEGF), rat TGF (rTGF), human TGF (hTGF), human amphiregulin (hA), vaccinia virus growth factor (VVGF), Shope fibroma virus growth factor (SVGF) and Myxoma virus growth factor (MVGF), human factor XII (hFXII) were aligned to allow maximal homology. *Dashes* represent spaces inserted for alignment and *dots* represent omitted amino acids. Invarient residues between the peptides are boxed. The VVGF, SVGF, and MVGF sequences have been truncated at the amino end (near residue 1). The letters *a, b,* and *c* show which cysteines should be matched.

ceptors because antibodies with differing specificity were used [7]. The primarily basal cell localization of the EGF/TGFα receptors implies that these receptors are only present on rapidly dividing cell populations in normal epidermis. Nothing could be further from the truth. EGF/TGFα receptors are also found in normal adult human skin on cells that do not rapidly divide [6]. In fact, the highest concentration of EGF receptors are found on the slowly dividing eccrine sweat duct cells that actively transport ions [6]. This distribution of EGF/TGFα receptors implies that the primary abnormality in psoriasis is not due to an intrinsic defect, but to factors regulating expression, availability, and activity of the EGF/TGFα receptor directly in the skin. In the "normal" or uninvolved epidermis of psoriatic patients the EGF/TGFα receptors are found by autoradiography and immunohistochemistry to be located primarily in the basal layers. In actively involved psoriatic epidermis the EGF/TGFα receptors are found throughout the epidermis in a pattern that indicates retention or persistence of the receptors into the parakeratotic stratum corneum [8]. Persistent expression of receptors may be dependent on the retention of the nucleus and nuclear membrane that may anchor structural proteins important in stabilizing cellular membrane receptors. In perilesional skin the number and pattern of EGF/TGFα receptors varies from slightly increased to almost total persistence of receptors. As the involved epidermis becomes more acanthotic and parakeratotic the receptors in the spinous cell and parakeratotic outer layers becomes more abundant [8].

Prior to treatment in the involved skin, the EGF receptors are increased two-to fourfold compared to uninvolved skin when measured based on the density of silver grains in autoradiographs made after the binding of ^{125}I-EGF. When treatment begins to be effective, the first detectable morphologic pattern to change is that of EGF/TGFα receptors. The receptors detected by anti-receptor antibodies or by ^{125}I-EGF binding begin to disappear as the stratum corneum begins to "normalize" and the parakeratosis begins to resolve. This decrease in the persistent expression of the EGF receptors did not appear to be dependent on the treatment modality because the same results were seen when psoriasis was effectively treated with UVB, PUVA, corticosteroids, or methotrexate. To date no consistent effect of retinoids on in vivo EGF receptor metabolism has been defined despite retinoids being an effective adjunctive therapy for patients with severe psoriasis. In vitro retinoic acid (RA) increases the number of EGF receptors expressed on some cell lines as much as sevenfold without altering their affinity for EGF [9]. In vivo PUVA induces an inhibition of EGF binding in a UVA dose and psoralen concentration-dependent manner and at higher doses UVA alone also induced inhibition of EGF binding [10]. How other agents affect the receptor binding and activity is not known, but these effects are likely to be mediated through multiple nuclear and cytoplasmic biochemical events. The persistent but reversible expression of EGF/TGFα receptors is not specific for psoriasis, as this pattern was also seen in newborn epidermis, paraneoplastic skin lesions, and some benign acanthotic dermatoses.* Similarly, persistent expression of membrane receptors such as low density lipoprotein (LDL) receptors was also detected throughout the involved psoriatic epidermis, indicating that the EGF/TGFα receptor defect is not unique [12]. Whether these multiple defects in cell membrane receptor expression are due to defects in the regulation of internalization, lysosomal degradation, or over-expression of the number of receptors is unclear. Preliminary data by Elder et al [13] concerning receptors is unclear and indicate that the increase in EGF receptors is not due to increased levels of specific mRNA for the receptor, so that defective internalization and degradation mechanisms appear more likely. No data are available for the LDL receptor or other receptors, so this issue is not settled.

In skin biopsies from sites of active proliferation of normal human skin and various skin diseases, an overall pattern emerges. The EGF/TGFα receptor persists in epidermis that retains its parakeratotic features such as in psoriasis and even normal mucosa. This generalization may be too broad because the EGF receptor distribution can vary even in uniform appearing tumors. For example, in mature, slowly growing seborrheic keratoses, which are a benign expansion of basaloid keratinocytes, EGF receptors can be found primarily confined to the basal and suprabasilar keratinocytes. However, in seborrheic keratoses, which are clinically documented to be actively growing, the EGF/TGFα receptors are found throughout the epidermis in the same pattern as that seen in active lesions of psoriasis and in newborn foreskin epidermis.

EGF/TGFα RECEPTOR REGULATION

If locally produced EGF or TGFα activates its receptor to affect keratinocyte proliferation/differentiation, what factors produced by other cell types might affect the EGF receptor? Elder et al found that the mRNA for TGFα but not EGF was increased in active psoriatic lesions [13]. The possibility of TGFα increasing not only the endogenous production of TGFα in affected keratinocytes but also inducing increased expression of the common EGF/TGFα receptor seemed likely. However, preliminary data suggest that message levels for the EGF receptor are not increased in psoriasis [13]. Certainly EGF binding and receptor activity may be affected by factors when the skin is injured in the Koebner reaction or isomorphic phenomenon. Wound macrophages express messenger RNA transcripts for TGFα, TGFβ, and PDGF [14]. Platelets contain EGF, TGFα, and TGFβ as well as a number of bioactive molecules such as PDGF, which may regulate EGF receptor binding and kinase activity [15]. Because TGFβ is synergistic with either EGF or TGFα to induce certain cellular effects, the benign overgrowth of keratinocytes in psoriatic plaques may have an in vitro correlate. TGFβ is both a growth stimulatory molecule for fibroblasts and a growth inhibitory molecule for human keratinocytes [15]. TGFβ modulates the high affinity form of the EGF/TGFα receptor [14]. Whether psoriatic keratinocytes abnormally respond to TGFβ as

they do to IFN (Nickoloff, this edition) is unknown. Because EGF increases calcium levels in treated cells [16] and EGF receptor activity may be altered by the calcium dependent protein kinase C (PKC), an obvious question is how does EGF metabolism interact with PKC and phosphatidyl inositol (PI) turnover [16]. The data are unclear, except that EGF receptors are involved in phospholipase C activation [17], are abnormal in psoriasis [8] (as is PKC metabolism [18]), and are degraded by a calcium-activated protease [19]. Because other calcium-related enzymes, such as calmodulin, are also abnormally regulated in psoriasis, it is unclear whether these are epiphenomena or pathogenetic events. Finally, the presence and distribution of the EGF receptor in viral skin diseases can either be increased or decreased depending upon the type of virus involved.* Aggravation of psoriasis by concomitant viral infections such as the AIDS virus (Human Immunodeficiency Virus, type III) is well-documented and may also reflect changes mediated by the EGF/TGFα receptor [8, 20]. Whether factors released by the dermal dendritic cell that may be important in the pathogenesis of AIDS and psoriasis also affect the metabolism of the EGF/TGFα receptor is of interest.

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